However, the detailed mechanism of the switching between the states has not been fully understood. Computationally, sampling the conformation space is the key limitation. We therefore employ the ''weighted ensemble'' (WE) simulation technique, a parallel, enhanced sampling method for characterizing non-equilibrium and equilibrium processes that would be impractical to observe using brute force simulation. Because it yields an ensemble of transition events, WE provides a unique lens for examining the mechanism (i.e., the time sequence of intermediate states), including possible heterogeneity, of conformational switching in all-atom ADK.

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Crosstalk between Hormone and DNA Binding Domains in Estrogen Receptor Alpha: An Energy Landscape Approach

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Estrogen receptor (ER) alpha is a hormone-activated transcription factor, which comprises of a highly conserved structural organization including a functional hormone binding domain (HBD) and a DNA binding domain (DBD). The mechanisms by which HBD and DBD interact in the regulation of hormone signaling are still unknown. Here, we apply a recently developed coarse-grained (CG) simulation pipeline to characterize the energy landscape of HBD-DBD interactions. First, CG models are built on HBD and DBD crystal structures and on their electrostatic and hydrophobic interactions. Second, ER molecular dynamics simulations are implemented and the sampling is enhanced by a push-pull-release strategy to comprehensively search for different HBD-DBD orientations. Finally, simulated ER configurations are projected onto a 3D energy globe and energetically stable conformations are identified. We found that among five identified ER conformations, four of them adopt very tight HBD-DBD packing utilizing a critical C-terminal helix (H12) as a key mediator at the domain interfaces, while the other takes a rather extended conformation and forms loose HBD-DBD contacts at a distant site. A closer examination also shows that while the binding sites from HBD for the four compact conformations are close in proximity, the DBD sites vary in contact residues and result into different HBD-DBD orientations. Detailed analyses on decomposing interfacial energies show that the molecular factors stabilizing these assembled conformations are from hydrophobic instead of electrostatic interactions. Taken together, these CG simulations and analyses reveal multiple distinct ER conformations that are accessible on the energy landscape and provide insight about HBD-DBD crosstalk into ER activation.

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Non-Equilibrium Fluctuation Theorems, Redundant Paths in Proteins, and Elucidating Conformational Changes by Single-Residue Perturbations

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Recently we have put forward the efficacy of single residue perturbations for a protein to populate its alternative conformational states[1-3]. In this work, we explain why mutation or protonation of a single-residue can create substantial conformational changes. First, we introduce a methodology which couples the network-based perturbation-response scanning (PRS) technique[4] with repeated constant force-steered molecular dynamics (CF-SMD)[5] simulations. Second, we utilize Crooks' fluctuation theorem[6] to measure the free-energy differences between conformational states to classify the circumstances under which a mutation or protonation achieves population shifts. Finally, we trace the relationship between the non-equilibrium trajectories(NET) from CF-SMD and redundant pathways in residue networks[1], and employ this relationship in interpreting the outcomes of the fluctuation theorems so as to ferret out the degree of collectivity in residue fluctuations.

Here are the procedure and sample results for calmodulin: (i)PRS takes the most-populated calmodulin conformation(3cln) and identifies E31whose directional-sensitive perturbations results in positional changes that yields the best overlap with considerably less populated states[7,2];(ii) through extensive MD simulations, E31A mutation reproduces structures consistent with those from NMR experiments[8];(iii) repeated CF-SMD runs for E31A produce NET between the two states, while those for E31 protonation do not;(iv) via Crooks' fluctuation theorem, we find the free-energy barrier between the two states to be +0.60kcal/mole for E31A. E31A MD runs reproduce conformational changes between states three orders of magnitude faster than in wild type[7]. Analysis of the NET for redundant paths reveals collectivity induced by strategically inserted point mutations.

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Dynamics of Extracellular Domains of Type I & II Cadherins: Disparity and Roles

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Cadherin-mediated adhesion plays a crucial role in multicellular organisms. Dysfunction of this adhesion system has major consequences in a number of pathologies, including cancer invasion and metastasis. While crystallographic structures for several cadherins show clear structural similarities, their relevant adhesive strengths vary and their mechanisms of adhesion between types I and II cadherin subfamilies are still unclear. The cadherin extracellular region is considered one of the dominant factors governing its adhesive properties, and unraveling its mechanical properties should bring a better understanding of the adhesion mechanism. Here, stretching of cadherins was explored experimentally by atomic force microscopy (AFM), and computationally by steered molecular dynamics (SMD) simulations, where the dominance of partial unfolding of the ectodomains was observed. SMD simulations on strand-swapping cadherin dimers displayed similarity in binding strength, suggesting contributions of other mechanisms to explain the strength differences of cell adhesion in vivo. Systematic simulations on the unfolding of the extracellular domains of types I and II cadherins revealed diverse pathways. However, at the earliest stage, a remarkable similarity in unfolding was observed for the various type I cadherins that was distinct from that for type II cadherins. This likely correlates positively with their distinct adhesive properties, suggesting that the initial forced deformation in type I cadherins may be involved in cadherin-mediated adhesion.

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Atomic Model Building from Low Resolution Electron Microscope Images of Integrin

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We have developed a computational approach to build an atomic model from an electron microscope (EM) image of proteins. In this approach, many atomic models are built first by deforming the X-ray crystal structure of the protein using a computational technique. Then, projection images of each atomic model to many different directions are generated. Finally, they are compared to the EM image. The atomic models with the projection images similar to the EM image are regarded as the candidates for the atomic structure of the protein from which the EM image was obtained.

This approach was applied to the EM images of integrin. Integrin is a membrane protein with a huge extracellular domain, and participates in cell-cell and cell-extracellular-matrix interactions. A group of integrins is known to perform a large-scale conformational change when the protein is activated.

The X-ray crystal structure of integrin was solved for the closed conformation, while many EM images were obtained for the open conformation. The difference of the two conformations is so large that it is difficult to build the atomic model for the EM conformations by deforming the X-ray crystal structure using conventional computational techniques. Thus, we have used the coarse-grained model of the X-ray crystal structure to simulate the large-scale conformational change.

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Using Hybrid Methods to Refine a Complete Atomic Structure of the Human Integrin αIIbβ3 Ectodomain into a Low-Resolution Electron Microscopy Map of the Intact Receptor in Nanodiscs

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The platelet integrin αIIbβ3 receptor plays a crucial role in thrombosis and hemostasis. A full-length atomic structure of the receptor is required to fully understand ligand binding and signaling. Guided by a 20.5 Å low-resolution electron microscopy (EM) map of the intact receptor in phospholipid bilayer nanodiscs, we used a combination of molecular dynamics flexible fitting (MDFF) and steered MD methods to refine a complete atomic structure of the human αIIbβ3 ectodomain into the EM maps. The complete ectodomain was built using the closed αIIbβ3 crystal structure and ab-initio methods to reconstruct missing loops. Several landmarks guided the MDFF, including binding of mAbs to specific domains as revealed by negative stain images. The characteristic shape of the αIIb β-propeller and the mAb binding sites furnished